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(54) Title: VACCINE COMPOSITION CONTAINING ADJUVANTS

(57) Abstract

The present invention provides vaccine compositions comprising 3 De-O-acylated monophosphoryl lipid A and QS21. The vaccines compositions are potent inducers of CTL and γ IFN responses.

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VACCINE COMPOSITION CONTAINING ADJUVANTS

The present invention relates to novel vaccine formulations, to methods of their production and to their use in medicine. In particular, the present invention relates to vaccines containing QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina, and 3 De-O-acylated monophosphoryl lipid A (3 D-MPL).

3 De-O-acylated monophosphoryl lipid A is known from GB2220 211 (Ribi). Chemically it is a mixture of 3-deacylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem Montana.

QS21 is a Hplc purified non toxic fraction of a saponin from the bark of the South American tree Quillaja saponaria molina and its method of its production is disclosed (as QA21) in US patent No. 5,057,540.

The present invention is based on the surprising discovery that formulations containing combinations of QS21 and 3 D-MPL synergistically enhance immune responses to a given antigen.

For example a vaccine formulation of the malarial antigen, RTS, S in combination with 3D-MPL and QS21 results in a powerful synergistic induction of CS protein specific cytotoxic T lymphocyte (CTL) response in the spleen.

RTS is a hybrid protein comprising substantially all the C-terminal portion of the circumsporozoite (CS) protein of P.falciparum linked via four amino acids of the $preS_2$ portion of Hepatitis B surface antigen to the surface (S) antigen of hepatitis B virus. It's full structure is disclosed in co-pending International Patent Application No. PCT/EP92/02591, published under Number WO 93/10152 claiming priority from UK patent application No.9124390.7. When expressed in yeast RTS is produced as a lipoprotein particle, and when it is co-expressed with the S antigen from HBV it produces a mixed particle known as RTS,S.

The observation that is possible to induce strong cytolytic T lymphocyte responses is significant as these responses, in certain animal models have been shown to induce protection against disease.

The present inventors have shown that the combination of the two adjuvants QS21 and 3D-MPL with the recombinant particulate antigen RTS,S results in a powerful induction of CS protein specific CTL in the spleen. QS21 also enhances induction of CTL on its own, while 3D-MPL does not. The combination can be said to act in a synergistic way, because it has an effect that is larger than the sum of the separate effects of each adjuvant. The synergy between these two adjuvants for CTL induction is a surprising observation which has important implications for the use of recombinant molecules as vaccines for induction of CTL mediated immunity.

Induction of CTL is easily seen when the target antigen is synthesised intracellularly (e.g. in infections by viruses, intracellular bacteria, or in tumours), because peptides generated by proteolytic breakdown of the antigen can enter the appropriate processing pathway, leading to presentation in association with class I molecules on the cell membrane. However, in general, pre-formed soluble antigen does not reach this processing and presentation pathway, and does not elicit class I restricted CTL. Therefore conventional non-living vaccines, while eliciting antibody and T helper responses, do not generally induce CTL mediated immunity. The combination of the two adjuvants QS21 and 3D-MPL can overcome this serious limitation of vaccines based or recombinant proteins, and induce a wider spectrum of immune responses.

CTL specific for CS protein have been shown to protect from malaria in mouse model systems (Romero et al. Nature 341:323 (1989)). In human trials where volunteers were immunised using irradiated sporozoites of P. falciparum, and shown to be protected against subsequent malaria challenge, induction of CTL specific for CS epitopes was demonstrated (Malik et al. Proc. Natl. Acad. Sci. USA 88:3300 (1991)).

The ability to induce CTL specific for an antigen administered as a recombinant molecules is relevant to malaria vaccine development, since

the use of irradiated sporozoites would be impractical, on the grounds of production and the nature of the immune response.

In addition to malaria vaccines, the ability to induce CTL responses would benefit vaccines against herpes simplex virus, cytomegalovirus, human Immunodeficiency virus, and generally all cases where the pathogen has an intracellular life stage.

Likewise, CTL specific for known tumour antigens could be induced by a combination of a recombinant tumour antigen and the two adjuvants. This would allow the development of anti cancer vaccines.

In certain systems, the combination of 3D-MPL and QS21 have been able to synergistically enhance interferon γ production. The present inventors have demonstrated the synergistic potential of 3D-MPL and QS21 by utilising a herpes simplex antigen known as gD_2t. gD_2t is a soluble truncated glycoprotein D from HSV-2 and is produced in CHO cells according to the methodology Berman et al. Science 222 524-527.

IFN-γ secretion is associated with protective responses against intracellular pathogens, including parasites, bacteria and viruses. Activation of macrophages by IFN-γ enhances intracellular killing of microbes and increases expression of Fc receptors. Direct cytotoxicity may also occur, especially in synergism with lymphotoxin (another product of TH1 cells). IFN-γ is also both an inducer and a product of NK cells, which are major innate effectors of protection. TH1 type responses, either through IFN-γ or other mechanisms, provide preferential help for IgG2a immunoglobulin isotypes.

Glycoprotein D is located on the viral envelope, and is also found in the cytoplasm of infected cells (Eisenberg R.J. et al J. of Virol. 1980 35 428-435). It comprises 393 amino acids including a signal peptide and has a molecular weight of approximately 60kD. Of all the HSV envelope glycoproteins this is probably the best characterized (Cohen et al. J. Virology 60 157-166). In vivo it is known to play a central role in viral attachment to cell membranes. Moreover, glycoprotein D has been shown to be able to elict neutralizing antibodies in vivo (Eing et al. J. Med Virology 127: 59-65). However, latent HSV2 virus can still be reactivated

and induce recurrence of the disease despite the presence of high neutralizing antibodies titre in the patients sera. It is therefore apparent that the ability to induce neutralizing antibody alone is insufficient to adequately control the disease.

In order to prevent recurrence of the disease, any vaccine will need to stimulate not only neutralizing antibody, but also cellular immunity mediated through T-cells, particularly cytotoxic T-cells.

In this instance the gD_2t is HSV2 glycoprotein D of 308 amino acids which comprises amino acids 1 though 306 of the naturally occurring glycoprotein with the addition of Asparagine and Glutamine at the C terminal end of the truncated protein. This form of the protein includes the signal peptide which is cleaved to yield a mature 283 amino acid protein. The production of such a protein in Chinese Hamster ovary cells has been described in Genentech's European patent EP-B-139 417.

The mature truncated glycoprotein D (rgD2t) or equivalent proteins secreted from mammalian cells, is preferably used in the vaccine formulations of the present invention.

The formulations of the present invention are very effective in inducing protective immunity in a genital herpes model in guinea pigs. Even with very low doses of antigen (e.g. as low as 5 μg rgD2t) the formulations protect guinea pigs against primary infection and also stimulate specific neutralising antibody responses. The inventors, utilising formulation of the present invention, have also demonstrated Effector cell mediated responses of the TH1 type in mice.

Accordingly, the present invention provides a vaccine or pharmaceutical formulation comprising an antigen in conjunction with 3 Deacylated monophosphoryl lipid A and QS21. Such a formulation is suitable for a broad range of monovalent or polyvalent vaccines.

Preferably the vaccine formulations will contain an antigen or antigenic composition capable of eliciting an immune response against a human or animal pathogen, which antigen or antigenic composition is derived from HIV-1, (such as gp120 or gp160), any of Feline Immunodeficiency virus,

human or animal herpes viruses, such as gD or derivatives thereof or Immediate Early protein such as ICP27 from HSV1 or HSV2, cytomegalovirus ((esp Human)(such as gB or derivatives thereof), Varicella Zoster Virus (such as gpI, II or III), or from a hepatitis virus such as hepatitis B virus for example Hepatitis B Surface antigen or a derivative thereof, hepatitis A virus, hepatitis C virus and hepatitis E virus, or from other viral pathogens, such as Respiratory Syncytial virus, human papilloma virus or Influenza virus, or derived from bacterial pathogens such as Salmonella, Neisseria, Borrelia (for example OspA or OspB or derivatives thereof), or Chlamydia, or Bordetella for example P.69, PT and FHA, or derived from parasites such as plasmodium or Toxoplasma.

The formulations may also contain an anti-tumour antigen and be useful for immunotherapeutically treating cancers.

The formulation may also be useful for utilising with herpetic light particles such as described in International Patent Application No. PCT/GB92/00824 and, International Patent Application No. PCT/GB92/00179.

Derivatives of Hepatitis B Surface antigen are well known in the art and include, inter alia, those $PreS_1$, $PreS_2$ S antigens set forth described in European Patent applications EP-A-414 374; EP-A-0304 578, and EP 198-474.

In a further aspect of the present invention there is provided a vaccine as herein described for use in medicine.

The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1; preferably 1 : 5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5:1 to 1:1 3D MPL: QS21. Typically for human administration QS21 and 3D MPL will be present in a vaccine in the range 1 µg - 100 µg, preferably 10 µg - 50 µg per dose. Often the vaccine will not require any specific carrier and be formulated in an aqueous or other pharmaceutically acceptable buffer. In some cases it may be advantageous that the vaccines of the present invention will further contain alum or be presented in an oil in water emulsion, or other suitable

vehicle, such as for example, liposomes, microspheres or encapsulated antigen particles.

Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 μg of protein, preferably 2-100 μg, most preferably 4-40 μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunisation adequately spaced.

The formulations of the present invention maybe used for both prophylatic and therapeutic purposes.

Accordingly in one aspect, the invention provides a method of treatment comprising administering an effective amount of a vaccine of the present invention to a patient.

Examples

1.0 Synergy between 3D MPL and QS21 for induction of Interferon γ secretion.

In order to test the ability of 3D MPL and QS21 based adjuvant formulations of rgD2t, to induce effector cell mediated immune responses, groups of Balb/c mice were vaccinated, and their draining lymph node cells tested for IFN-γ secretion as described below.

1.1 rgD2t formulations

This experiment compared three adjuvant formulations:

i) rgD2t in 3D-MPL
ii) rgD2t in QS21
iii) rgD2t in 3D-MPL/QS21

These formulations were made up as follows. rgD2t was produced in CHO cells and corresponds to the mature 1-283 amino acids of HSV-2 gD and is produced according to the methodology of Berman (supra) and EP 0139417.

* rgD2t / 3D-MPL

5 µg of rgD2t/dose are incubated 1h, under agitation,at room temperature, then mixed with a 3D-MPL suspension (25 µg/dose). The volume is adjusted to 70 µl/dose using a sodium chloride solution (5M, pH 6.5 ± 0.5) and water for injection to obtain a final concentration of 0.15M sodium chloride. pH is kept at 6.5 ± 0.5.

* rgD2t/QS21

5 µg rgD2t/dose are incubated 1h at room temperature under agitation The volume is adjusted using sodium chloride solution (5M, pH 6.5 ± 0.5) and water for injection to 70µl. QS21 (10 µg/dose) is then added. pH is kept at 6.5 ± 0.5.and sodium chloride final concentration at 0.15M.

* rgD2t/3D-MPL / QS21.

5 µg rgD2t/dose are incubated 1h at room temperature under agitation. 3D-MPL (25 µg/dose) is added as an aqueous suspension. The final volume of 70µl is completed by addition of an aqueous solution of QS21 (10 µg/dose) and the pH kept at 6.5 ± 0.5 and the sodium chloride concentration at 0.15M.

1.2 IMMUNISATION

Mice were injected into the hind footpads with 35 µL/footpad of formulation. Thus each mouse received 70 µL. Immunisation were on days 0, and 14. Animals were sacrificed on day 21.

1.3 INTERFERON γ ASSAYS

Popliteal lymph node cells from immunised mice were stimulated in vitro using rgD2t at 10, 1, 0.1, 0 µg/ml. Triplicate cultures (200 µl volumes) were set up in round bottom 96-well microtiter plates, using 2 x 10^5 responder cells and 2 x 10^5 irradiated (3000 rad) syngeneic naive spleen cells. Culture medium was RPMI 1640 with 10% foetal calf serum. Aliquots of 100 µl of culture medium from each replicate were harvested and pooled for IFN-γ determinations. Cultures were assayed at 72 hours. For all assays, a control group using ConA (Boehringer Mannheim) at 5 µg/mL was included. This was always positive.

Secretion of IFN-γ was determined using a commercial ELISA assay manufactured by Holland Biotechnology (distributed by Gibco). Assays were carried out on 100 µl of pooled supernatant from triplicate wells.

Secretion of IFN-γ above the assay background of 50 pg/µl was observed in all three formulation groups (see Table). In addition, a synergistic effect between QS21 and 3D-MPL was observed. While each adjuvant on its own induced cells capable of secreting IFN-γ in response to rgD2t, their combination induced more than twice the sum of individual responses.

1.4 Results

Synergy between QS21 and 3D-MPL for induction of IFN-γ secretion.

Immunization:		QS21/3D-MPL rgD2t	QS21 rgD2t	3D-MPL rgD2t
In vitro	10.0	1351	1105	515
stimulation	1.0	914	116	192
(μg/mL gD2t):	0.1	335	<50	143
	0.0	101	<50	139

IFN-γ is expressed in pg/mL.

The table clearly shows that the combined vaccine induces IFN-γ-secretion in a synergistic manner.

2.0 Synergy Between 3D MPL and QS21 for the induction of CTLs

In order to test the ability of RTS,S particles in 3D MPL and QS21 based adjuvant formulations to induce CTLs, groups of B10.BR mice were immunised and their spleen cells stimulated in vitro and tested in cytotoxicity assays on L cells expressing the CS protein.

2.1 Formulation of RTS,S particles.

RTS,S particles were formulated in three different compositions:

1. RTS,S particles ((10μg) with QS21 (10μg) and 3D-MPL (25μg);
2. RTS,S particles ((10μg) with QS21 (10μg);
3. RTS,S particles ((10μg) with 3D-MPL (25μg);

The formulations were made up as follows:

RTS, S/3 D MPL

10 µg of RTS, S particles/dose was incubated at room temperature under agitation then mixed with a 3D MPL aqueous suspension (25µg/dose). The volume is then adjusted to 70 µl/dose using water for injections and a sodium chloride solution (5N, pH 6.5 ± 0.5) to reach a final concentration of 0.15M sodium chloride (pH is kept at 6.5 ± 0.5).

RTS,S /QS21

10µg of RTS, S particles/dose incubated 1h. at room temperature under agitation. The volume is adjusted using water for injection and a sodium chloride solution (5N, pH 6.5 ± 05) and completed to a final volume of 70µ l/ dose with an aqueous solution of QS21 (10µg/dose). pH is kept at 6.5 ± 0.5 and sodium chloride final concentration at 0.15M.

RTS,S / 3 D MPL / QS21

10 µg of RTS,S particles / dose are incubated 1h. at room teperature under agitation then mixed with a 3D MPL (aqueous suspension (25µg/dose) - The volume is then adjusted with water for injection and a sodium chloride solution (5D pH 6.5 ± 0.5). The final volume is completed by addition of an aqueous solution of QS21 (10µg/dose). pH is kept at 6.5 ± 0.5, and sodium chloride final concentration at 0.15 M.

2.2 Immunisation of mice with RTS,S particles

Four to six week old female mice of the strain B10.BR (H-2^k) were purchased from IFFA CREDO (France). Groups of 3 animals were immunised by intra foot-pad injection of 35 µL of antigen formulation into each hind limb. The animals were boosted with a second equal dose of antigen injected two weeks later.

2.3. In vitro stimulation on anti CS CTL

Two weeks after the boost, spleen cells were harvested and stimulated in vitro using syngeneic fibroblasts transfected with the P. falciparum circumsporozoite protein gene (7G8 clone). These CS-transfectant cells

have been described in the paper by Kumar, S. et al. (1988), Nature 334:258-260.

The cultures were established in RPMI 1640 medium supplemented with 10% of heat inactivated foetal calf serum and usual additives, in conditions well known to those of skill in the art.

Responder cells were cultured at a concentration of 10^6 cells/mL in the presence of 10^5 CS-transfectants per mL. To prevent proliferation of CS-transfectant cells, these were irradiated using a dose of 2×10^4 rad. The cultures were fed by replacing 1/2 of culture medium on day 3 and 6, and tested for cytolytic activity on day 7.

2.4. Cytotoxicity assay for anti-CS CTL

Responder cell cultures were harvested, washed, and mixed at ratios varying from 100:1 to 0.3:1 with a constant number of 2000 target cells, in volumes of 200 µL of medium in V-bottom 96-well plates.
Target cells were syngeneic fibroblast cells that had been labelled with ^{51}Cr.

Two different types of target cells were used:

1. L cells
2. CS transfected L cells

These are described in: Kumar, S. et al. (1988), Nature 334:258-260.

The assay was incubated for 6 hours at 37°C, then the amount of radioactivity released into the supematant by lysis of target cells was determined. Cytolytic activity is expressed as % specific lysis:

Results:

Target cells:	Effector: target ratio	% Specific lysis by formulation: 1. RTS,S/ QS21/ 3D-MPL	2. RTS,S/ QS21/	3. RTS,S/ 3D-MPL
CS transfected L cells	100	58	17	1
	30	53	10	0
	10	47	5	1
	3	27	1	0
	1	11	0	0
	0.3	2	-2	-1
L cell	100	3	-2	5
	30	-2	1	4
	10	0	-1	2
	3	0	3	4
	1	-1	4	2
	0.3	3	1	2

Immunisation of B10.BR mice with RTS,S adjuvanted with QS21 and 3D-MPL (formulation #1) induced in the spleen high levels of CTL specific for the circumsporozoite component of RTS,S. Immunisation with RTS,S particles adjuvanted with QS21 (formulation #2) also induced CTL in the spleen, but only at about 1/30th of the levels given by formulation #1. RTS,S with 3D-MPL (formulation #3) did not induce CTL.

Since the target cells used in this assay do not express MHC class II molecules, the effector cells can be assumed to be $CD8^+$, class I restricted CTL.

3. Other formulation

Hepatitis B Surface Antigen, Alum 3D-MPL and QS21.

The preparation of Hepatitis B Surface antigen (HBsAg) is well documented. See for example Harford et al Develop. Biol. Standard 54 p125 (1983), Gregg et al Biotechnology 5 p479 (1987) EP-A-O 226 846 and EP-A-299 108 and references therein.

3D-MPL was obtained from Ribi Immunochem, QS21 was obtained from Cambridge Biotech, and Aluminium hydroxide was obtained from Superfos (Alhydrogel).

A number of different formulations were made up for studies of cell mediated immunity in mice and for studies in Rhesus monkeys.

3.1 Formulation 1 was made up in phosphate buffer (pH 6.8) to comprise the following per 60 µl dose.

20 µg	HBsAg
30 µg	$Al(OH)_3$
30 µg	3D - MPL
10 µg	QS 21
10 mM	PO_4^{3-}
0.15 M	NaCl

The formulation was made up in the following manner. 20µg HBsAg/dose was incubated with $Al(OH)_3$ for one hour at room temperature with gentle shaking. 3D-MPL was added as an aqueous suspension, and the formulation completed by the addition of QS21, phosphate buffer and sodium chloride and incubated for one hour at room temperature. The final formulation had a pH of between 6.5 and 7.0 and used for foot pad studies in mice.

3.2 Formulation 2 was made up in a phosphate buffer (pH6.8) to comprise the following per 200 µl dose.

1 µg	HBsAg
100 µg	$Al(OH)_3$
50 µg	3D-MPL
20 µg	QS 21
10 mM	PO_4^{3-}
0.15 M	NaCl

The formulation was made up in the following manner. HBsAg and $Al(OH_3)$ were incubated together for one hour at room temperature with

gentle shaking. The formulation was completed by the addition of $Al(OH)_3$, 3D-MPL as an aqueous suspension and QS21, with phosphate buffer and sodium chloride solution and incubated again for thirty minutes. The pH of the formulation was kept between 6.5 and 7.0 and used for Humoral immunity studies in mice.

3.3 Formulation 3 was made up in a similar manner, in a phosphate buffer (pH6.5 - 7.0) to contain the following per 1 ml dose :

10 µg	HBsAg
500 µg	$Al(OH)_3$
50 µg	3D-MPL
10 µg	QS 21

The formulation was used for monkey studies.

4. Conclusions

The combination of the two adjuvants QS21 and 3D-MPL with the recombinant particulate antigen RTS,S resulted in a powerful induction of CS protein specific CTL in the spleen. QS21 enhances induction of CTL on its own, while 3D-MPL does not. The combination can be said to act in a synergistic way, because it has an effect that is larger than the sum of the separate effects of each adjuvant. The synergy between these two adjuvants for CTL induction is a surprising observation which supports our observation of synergy between QS21 and 3D-MPL for induction of T cells capable of secreting IFN-γ in response to stimulation with the soluble recombinant protein gD2t. This finding has important implications for the use of recombinant molecules as vaccines for induction of CTL mediated immunity, since the combination of the two adjuvants QS21 and 3D-MPL can overcome this serious limitation of vaccines based on recombinant proteins, and induce a wider spectrum of immune responses than hitherto.

The mouse cell mediated immunogenicity data show that QS21 based formulations of rgD2t induce a significant synergistic TH1 type T cell response (IFN-γ secretion).

Such TH1 type T cells have been shown to be involved in induction of delayed type hypersensitivity responses in mice. Our own data in prophylaxis of HSV disease show that concomitant induction of neutralizing antibody titers and antigen specific DTH responses affords the best protection against herpes simplex disease.

Put together, these data suggested to us that QS21 formulations of rgD2t may be effective in inducing a protective response against HSV disease. The data presented show an unexpected synergistic effect between 3D Monophosphoryl lipid A and QS21, in inducing IFN-γ secreting antigen specific T cells. Such a synergy may translate in improved ability to induce a protective response against HSV disease, and indeed these formulations are effective in protecting against disease in guinea pigs.

Claims

1. A vaccine composition comprising an antigen and/or antigenic composition, QS21 and 3 De-O-acylated monophosphoryl lipid A (3D-MPL).

2. A vaccine as claimed in claim 1 wherein the ratio of QS21:3D-MPL is from 1:10 to 10:1.

3. A vaccine composition as claimed in claim 1 or 2 capable of invoking a cytolytic T cell response in a mammal to the antigen or antigenic composition.

4. A vaccine composition as claimed in any of claims 1 to 3 capable of stimulating interferon γ production.

5. A vaccine composition as claimed in any of claims 1 to 4 wherein the ratio of QS21:3D-MPL is from 1:1 to 1:2.5.

6. A vaccine composition as claimed herein comprising an antigen or antigenic composition derived from any of Human Immunodeficiency Virus, Feline Immunodeficiency Virus, Herpes Simplex Virus type 1, Herpes Simplex virus type 2, Human cytomegalovirus, Hepatitis A,B,C or E, Respiratory Syncytial virus, human papilloma virus, Influenza virus, Salmonella, Neisseria, Borrelia, Chlamydia, Bordetella, Plasmodium or Toxoplasma.

7. A vaccine as claimed in any of claim 1 to 5 wherein the antigen is a tumour antigen.

8. Use of composition as defined in any of claims 1 to 5 for the manufacture of a vaccine for the prophylatic treatment of viral, bacterial, or parasitic infections.

9. Use of composition as defined in any of claims 1 to 5 for the manufacture of a vaccine for the immunotherapeutic treatment of viral, bacterial, parasitic infections or cancer.

10. A method of treating a mammal suffering from or susceptible to a pathogenic infection comprising the administration of a safe and effective amount of a composition according to any of claims 1 to 5.

11. A method of treating a mammal suffering from cancer comprising the administration of a safe and effective amount of a composition according to any of claims 1 to 5.

12. A process for making a vaccine composition according to claims 1 to 5 comprising admixing QS21 and 3D-MPL with an antigen or antigenic composition.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 93/01524

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K39/39; A61K39/00; // A61K39/245,A61K39/295

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[10]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	BIOTECHNOLOGY vol. 20, 1992, pages 431 - 449 ANTHONY C. ALLISON ET AL. 'IMMUNOLOGICAL ADJUVANTS AND THEIR MODE OF ACTION' see page 437, paragraph 19.6 - page 439 see page 441, paragraph 19.8 - page 442	1
A	J. ANIMAL SCIENCE vol. 68, 1990, pages 3742 - 3746 A. J. ROBERTS ET AL. 'ACTIVE IMMUNIZATION OF BEEF HEIFERS AGAINST LUTEINIZING HORMONE...' see page 3743; table 1	1

[10] Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 SEPTEMBER 1993	04. 10. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	REMPP G.L.E.

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/EP 93/01524

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claims 10-11 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.